Figure 1. *Response surface predictions for the survival of* S. typhimurium *exposed to ionizing radiation on freeze-dried pork* L. dorsi *muscle tissue rehydrated to various water contents.*

Figure 2. *Response surface predictions for the survival of* S. typhimurium *exposed to ionizing radiation on freeze-dried pork* L. dorsi *muscle tissue rehydrated with solutions having various levels of NaCl saturation. The solubility of NaCl is 35.71 g/100 ml* H_2O *at 5°C.*

By comparing measurements of detection time to those for equal populations of uninjured *S. typhimurium*, we estimate that 30, 33, 22, 21, and 27% of the cells surviving a radiation dose of 1.5 kGy were injured in pork rehydrated with salt solution concentrations of 0, 25, 50, 75, and 100% saturation, respectively. The radiation D_{10}-values for *S. typhimurium* in each of the meats are estimated to be 0.48
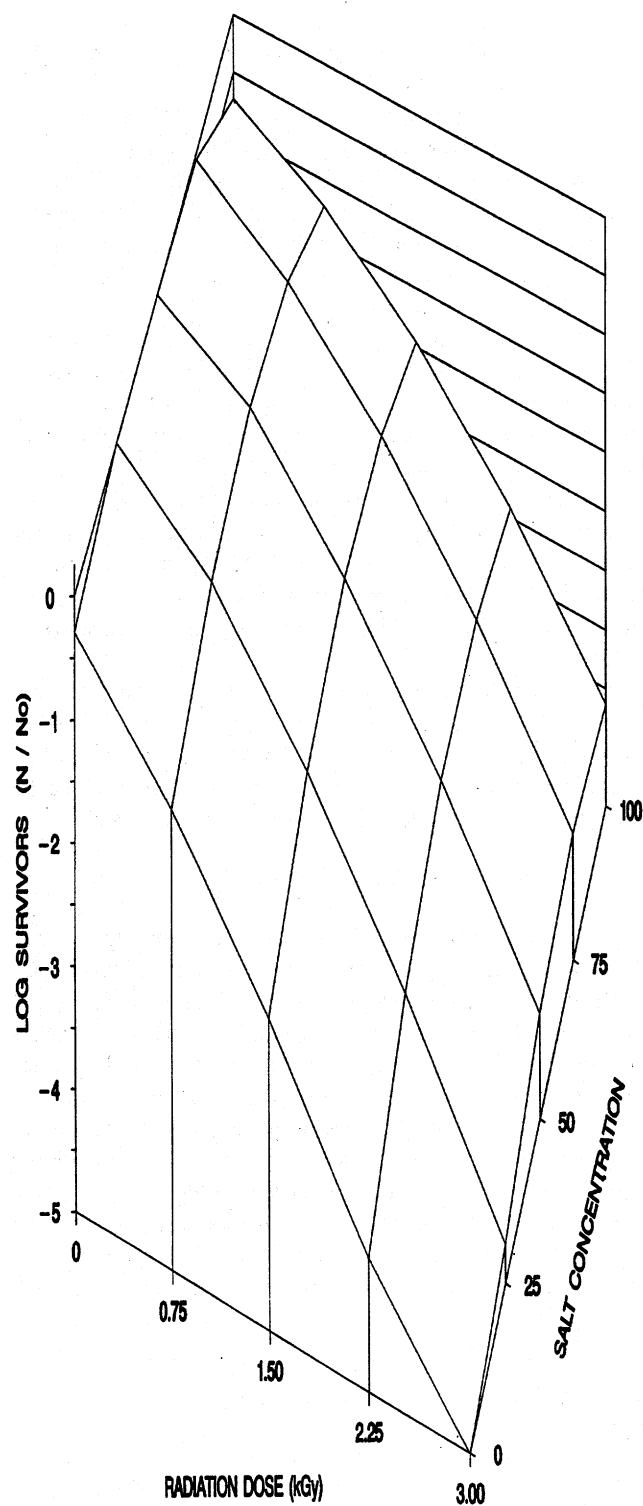
$\pm$ 0.03, 0.73 $\pm$ 0.05, 0.78 $\pm$ 0.04, 0.87 $\pm$ 0.05, and 0.72 $\pm$ 0.04 kGy in meat rehydrated with 0, 25%, 50%, 75%, and 100% NaCl saturation, respectively.

The appropriate response surface for the logarithm$_{10}$ of the number of surviving s^r as affected by the a$_w$ of the rehydrated freeze dried pork muscle is described by equation VI (Table 7).

Survival of S. typhimurium *in NaCl or sucrose solutions of equal a_w-value*

Survival of irradiated *S. typhimurium* ATCC 14028 in NaCl solutions was inversely dependent on the a_w and directly dependent on the molality of the solution (Table 6). The coefficient of correlation for the linear regression of the logarithm of the number of the CFU was slightly better for molality, r = +0.801, than for a_w, r = +0.784, of the suspending NaCl solution.

The coefficients of correlation for the linear regression of the logarithm of the number of CFU were also better for molality, r = +0.624, than for a_w, r = +0.596, of the suspending sucrose solution. The sucrose values obviously did not fit a linear regression model well.

Analysis of covariances for data from 0.85 a_w to 0.995 indicated that the regression of the logarithm of the number of CFU versus a_w differed (p < 0.0170) for the samples containing NaCl from those containing sucrose. The regressions against a_w and against molality did not differ (p > 0.05).

The correlation of injury with either a_w or the molality of the suspending medium was very poor (p > 0.05). Compared to the results obtained with the buffer controls (1.0 a_w), both sucrose and NaCl solutions protected the cells during irradiation better than did phosphate buffer.

DISCUSSION

The most striking result of this study was the dissimilarity of results obtained from adding either NaCl or sucrose to meat. If the increased survival of *S. typhimurium* in irradiated meat was due to reduced a_w, then any product that reduced the a_w to an equivalent level should produce the same results. Yet in our initial studies we observed no protection from adding sucrose to ground pork, whereas we did observe significant radiation protection from adding NaCl. We did not confirm the increased lethality when salt was present that was found by Matsuyama et al. *(15, 16)*. We observed that injury of the irradiated cells was reduced proportionately to the amount of NaCl added to pork except

TABLE 6. *Survival of* S. typhimurium *ATCC 14028 when irradiated (0.8 kGy at 5.0°C) in NaCl or sucrose solutions of various a_w-values.*

Water activity	Salt		Sucrose	
a_w	Log CFU/ml	% Injury	Log CFU/ml	% Injury
0.850	8.49	26	9.22	11
0.900	8.40	28	8.97	13
0.920	8.19	27	8.83	20
0.960	7.94	32	8.56	21
0.980	7.38	32	8.72	19
0.995	6.24	41	8.66	18
1.000[a]	6.09	49	6.92	53

The values reported are the means of two independent studies with each of the solutes. The inocula for the sucrose study were slightly greater than those for the NaCl study.
The solvent was 0.05 M potassium phosphate (pH 7.0).

TABLE 7. *Response-surface equations predicting the effects of gamma irradiation on the survival of* S. typhimurium *ATCC 14028 on mechanically deboned chicken meat (MDCM) or pork containing added NaCl, or on rehydrated freeze-dried pork, or on freeze-dried pork rehydrated with NaCl solutions.*

Equation I—NaCl added to MDCM (Effect of NaCl)
Log_{10} of the number of survivors (N/N_0) = -0.137 - 2.335 × kGy - 0.010 × % salt + 0.087 × kGy × % salt + 0.057 × kGy^2 + 0.004 × $salt^2$ $(100 R^2)$[a] = 95.2 p-values[b] are: 0.0001, 0.9395, 0.0093, 0.6003, and 0.7888.

Equation II—NaCl added to pork (Effect of NaCl)
Log_{10} of the number of survivors (N/N_0) = -0.137 - 1.799 × kGy + 0.009 × % salt + 0.099 × kGy × % salt - 0.192 × kGy^2 - 0.003 × $salt^2$ $(100 R^2)$ = 96.3 p-values[b] are: 0.0001, 0.9402, 0.0024, 0.0803, and 0.8135.

Equation III - Rehydrated freeze-dried pork
(Effect of water content)
Log_{10} of the number of survivors (N/N_0) = -0.160 - 0.149 × kGy - 0.013 × % water - 0.020 × kGy × % water - 0.259 × kGy^2 + 0.000 × % $water^2$ $(100 R^2$ = 97.0 p-values[c] are: 0.5860, 0.4679, 0.0001, 0.0035, and 0.2313.

Equation IV—Rehydrated freeze-dried pork (Effect of a_w)
Log_{10} of the number of survivors (N/N_0 = -6.309 + 0.162 × a_w + 1.274 × kGy - 0.026 × a_w × kGy - 0.278 × kGy^2 + 0.001 × a_w^2 $(100 R^2)$ = 97.1 p-values[d] are: 0.0836, 0.0069, 0.0001, 0.0016, and 0.1023.

Equation V—Freeze-dried pork rehydrated with NaCl solutions (Effect of NaCl)
Log_{10} of the number of survivors (N/N_0) = -0.291 - 1.144 × kGy + 0.026 × % NaCl + 0.006 × kGy × % NaCl - 0.210 × kGy^2 - 0.000 × $NaCl^2$ $(100 R^2)$ = 96.5 p-values[a] are: 0.0001, 0.0001, 0.0001, 0.0001, and 0.0001.

Equation VI—Freeze-dried pork rehydrated with NaCl solutions (Effect of a_w)
Log_{10} of the number of survivors (N/N_0) = - 54.347 + 0.262 × a_w + 1.206 × kGy - 0.023 × a_w × kGy - 0.210 × kGy^2 - 0.007 × a_w^2 $(100 R^2)$ = 94.7 p-values[d] are: 0.0001, 0.0752, 0.0025, 0.0011, and 0.0001.

[a] $(100 R^2)$: The coefficient of determination.
[b] The p-values for partial t-tests of the parameter estimates for kGy, % salt, kGy × % salt, kGy^2, and % $salt^2$.
[c] The p-values for partial t-tests of the parameter estimates are for kGy, % water, kGy × % water, kGy^2, and % $water^2$.
[d] The p-values for partial t-tests of the parameter estimates for a_w, kGy, a_w, × kGy, kGy^2, and a_w^2.

for the samples containing the largest amount, which seemed to be slightly toxic. Because of the many variables resulting from the addition of either NaCl or sucrose to meat or poultry, we chose to further test the concept that radiation resistance was indirectly related to the a_w of the suspending medium. In the experiments using NaCl and sucrose solutions with various a_w-values, both NaCl and sucrose provided radiation protection compared to the control buffer, but the radiation resistance in the NaCl solutions decreased with increased a_w. To a limited extent the same effect was noted in sucrose, but the fit of the linear regression model was poor. The regressions were not the same, thus it

Effects of NaCl, Sucrose, and Water Content on the Survival of *Salmonella typhimurium* on Irradiated Pork and Chicken

ABSTRACT

We investigated the effects of water content, activity, sodium chloride (NaCl) and sucrose contents on the survival of *Salmonella typhimurium* ATCC 14028 in irradiated mechanically deboned chicken meat (MDCM) and ground pork loin. The effects of NaCl and sucrose concentration were investigated by adding various amounts to MDCM or ground pork loin, or by rehydrating freeze-dried ground pork loin with NaCl solutions with various degrees of saturation. The effects of water content were investigated by rehydrating freeze-dried ground pork loin with different quantities of water. Inoculated samples were irradiated at 5°C *in vacuo* to various doses up to 6.0 kGy. Highly significant effects ($p < 0.01$) of water content, water activity (a_w) and NaCl content, but not of sucrose content, on the survival of *S. typhimurium* were identified. The failure of sucrose to provide the same protection for *S. typhimurium* in meat against radiation argues against reduced water activity being a primary mechanism of protection. The results indicate that the survival of foodborne pathogens on irradiated meats with reduced water content or increased NaCl levels may be greater than expected.

Key Words: Irradiation, pork, chicken, *Salmonella typhimurium*, salt, water

In a previous study we found that reducing the water content of bacon provided significant protection to thiamin when the bacon was irradiated *(28)*. Bruns and Maxcy *(3)* discovered that freeze-dried *Moraxella* spp. were much less sensitive to ionizing radiation and that radiation sensitivity was not temperature dependent. It has been known for many years that a_w of the suspending medium can have effects on the heat resistance of many bacteria, including *Salmonellae (8)*. Moussa *(17)* found radioresistance of stationary-phase cells of *S. typhimurium*, *Salmonella thompson*, and *Salmonella seftenberg* increased as a_w decreased; unfortunately, this reference gives no experimental details. Härnulv and Snygg *(9)* found that spores of *Bacillus subtilis* and *Bacillus stearothermophilus* were more radiation resistant under anoxic conditions at lower a_w in water vapor or in glycerol or glucose solutions.

Reference to a brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Okazawa et al. *(18)* and Matsuyama et al. *(15, 16)* reported that the lethality of ionizing radiation for vegetative cells of *Escherichia coli*, but not *B. subtilis* spores, was increased by the presence of NaCl during irradiation. They speculated that the increased lethality of radiation in the presence of NaCl might be due to the generation of chloride radicals. Anbar and Thomas *(1)* found evidence for the formation of a Cl^{-2} radical by reaction of NaCl with OH• radicals. Firstenberg-Eden et al. *(5)* found that *Moraxella* and *Acinetobacter* cells were more resistant to heat but not radiation inactivation when heated in meat containing 0.75% NaCl and 0.37% sodium tripolyphosphate salts than in meat without these salts. Ma and Maxcy *(12)* reported that the presence of up to 8% NaCl during irradiation did not alter the radiation resistance of *Moraxella* or *Acinetobacter*. We had noted, as had others, that the survival of several foodborne pathogens in irradiated meats was dependent on the temperature of the suspending medium; there was a sharp increase in survival at the freezing point for the meat *(22, 24-26)*. The increased radiation resistance of the bacterial cell thus corresponded to a change in the physical state of water. It is well known that the mobility of the hydroxyl radical is reduced below the freezing point *(23)*. Some of the observations reported above are contradictory, especially those due to the presence or absence of NaCl. Processed meat and poultry products frequently have reduced water content or a_w because of the presence of NaCl or sucrose. We theorized that the water content or its degree of organization in the suspending medium should affect the survival of foodborne pathogens and the retention of vitamins in meat or poultry when irradiated. Therefore, in this study we investigated the effects of water content and a_w in chicken or pork as influenced by NaCl or sucrose on the survival of *S. typhimurium* and in a companion study the retention of thiamin and Vitamin E in irradiated pork *(6)*.

MATERIALS AND METHODS

Culture and culture conditions

Salmonella typhimurium ATCC 14028 was obtained from the American Type Culture Collection, Rockville, MD, maintained on tryptic soy agar (TSA) (Difco Laboratories, Detroit,

MI), and cultivated aerobically in tryptic soy broth (TSB) (Difco) at 35°C. Culture identity and purity of this serovar were verified by Gram stain and biochemical reactions using the Vitek Automicrobic System® GNI card (bioMérieux Vitek, Inc., Hazelwood, MO) (10), and confirmed by serologic testing with polyvalent and individual O-group antisera (Fisher Diagnostics Salmonella Diagnostic Sera, Fisher Scientific Co., Orangeburg, NY). A streptomycin-resistant mutant of S. typhimurium (s^r), isolated and stability determined as described previously (27), was used for studies with non-sterile meat, as indicated below.

One milliliter from a 15 to 18-h culture of the appropriate strain incubated at 35°C in TSB was used to inoculate 100 ml of TSB in a 500-ml baffled shake flask. These cultures were incubated aerobically with shaking (150 rpm) at 35°C and harvested at 16 h by centrifugation. A ten-fold inoculum was prepared by resuspending the cells in 1/10 volume of Butterfield's phosphate (0.25 M potassium hydrogen phosphate [KH_2PO_4] adjusted to pH 7.2 with sodium hydroxide [NaOH]).

Microbiological assay

Samples were assayed for colony-forming units (CFU) in TSA after 24 h incubation at 35°C by standard pour-plate procedures with serial dilutions in sterile Butterfield's phosphate buffer. Assays for CFU of the streptomycin-resistant isolate were made in TSA containing 1.0 g/liter of streptomycin sulfate (Sigma Chemical Co., St. Louis, MO). Colony-forming units were counted with a Biotran II automated colony counter (New Brunswick Scientific, Edison, NJ) on three petri plates containing 30 to 300 colonies; the lower detection limit was 10 CFU/g.

Measurement of detection time and estimation of injured population

Impedance analyses were performed using the bioMérieux Vitek Bactometer. Plate counts were made of each culture. Samples (1.0 ml) for assay of detection time were withdrawn from the 10^{-2} dilution for plate counting and mixed with 9 ml of Wilkins-Chalgren Anaerobe Broth (Oxoid, Ltd., Basingstoke, Hampshire, England). Samples (2.0 ml) were placed in two assay wells of the Bactometer plate to determine detection time at 30°C. Detection times for uninjured cells were determined for a wide range of populations by using serial dilutions of the unirradiated control samples from each study. Standard curves were prepared by plotting CFU against detection times. From this standard curve a population estimate can be made for each detection time measured or vice versa. The difference between this value and that obtained from counts of CFU on pour plates represents the injured population. The assumption is that injured cells require more time to initiate the log-growth phase than do uninjured cells, as found by Mackey and Derrick (13). Because detection time depends on both the initial number of cells per unit volume and the lag time, we do not equate the detection time with lag time (14). Mackey and Derrick used conductance measurements to estimate the lag phase of injured S. typhimurium (14).

Irradiation

Samples were irradiated with a self-contained ^{137}Cs-gamma-radiation source with a dose rate of 0.114 kGy/min. The dose rate was established using National Physical Laboratory (Middlesex, United Kingdom) dosimeters. Variations in dose absorption were minimized by placing thin samples (approximately 2 mm thick) within a uniform portion of the radiation field. The total mass of 5-g samples undergoing irradiation at one time usually did not exceed 20 g. Samples were maintained within ± 0.5°C of the desired temperature by injecting the gas phase from liquid nitrogen into the irradiation chamber. Sample temperature was monitored continuously during irradiation.

Measurement of a_w

Water activity was measured on duplicate 5.0 g samples using the Rotronic Hygroskop DT (Rotronic Instrument Corp., Huntington, NY) using WA40th Sensors with integral water jackets. The temperature was maintained at 25° ± 0.05°C. Initial measurements were made with the instrument calibrated at 80% relative humidity (RH), and then the instrument was recalibrated immediately before measurement using reference saturated salt solutions as similar as possible to those of the actual samples. The following were used as reference saturated solutions: $ZnSO_4 \times 7H_2O$, 90% RH; $(NH_4)_2SO_4$, 81% RH; NaCl, 75% RH; and $Ca(NO_3)_2 \times 4H_2O$, 51% RH (11).

Measurement of moisture and ash content

Moisture content of meat samples was determined with the CEM Model AVC80 (CEM Corp., Matthews, NC) moisture computer according to the manufacturer's instructions. Ash content was determined with the CEM microwave ashing system -300 using the manufacturer's directions.

Preparation of sterile mechanically deboned chicken

Mechanically deboned chicken meat was obtained in 18-kg lots from a local manufacturer of poultry frankfurters. It was subdivided into 100 ± 0.05 g amounts, spread uniformly over an area of approximately 10 × 10 cm within polyethylene Stomacher 400 (Tekmar Co., Cincinnati, OH) bags and vacuum-sealed. These bags were themselves vacuum-sealed within Freshstuff (American National Can Company, Des Moines, IA) oxygen barrier pouches (oxygen transmission 0.6-0.8 cc/645 cm²/24 h at 3.5°C and 90% relative humidity). The replicate samples of MDCM were then rapidly frozen at -50°C. Sterile MDCM was prepared by gamma irradiating the vacuum-packaged product to a dose of 42 kGy at -40°C (12-D-value for Clostridium botulinum endospores in chicken) (2) and stored at -20° or lower until used.

Effect of addition of NaCl to sterile MDCM on the survival of S. typhimurium when irradiated

Sterile MDCM was mixed with 2, 4, 6, or 8% wt/wt of NaCl and inoculated with 8.65 $\log_{10}$ CFU/g of S. typhimurium ATCC 14028. Each well-mixed sample was aseptically subdivided into 5.0 ± 0.05 g samples, which were spread over an area of approximately 10 × 10 cm in sterile Stomacher 400 polyethylene bags and vacuum-sealed. Each sample bag was overbagged under vacuum in a Freshstuff barrier bag for additional protection during handling and to ensure a low oxygen concentration in the meat. A modified response-surface central composite design (Table 1), with two independent replicates, was used to examine the effects of irradiation doses of 0, 0.75, 1.50, 2.25, and 3.0 kGy at 5.0 ± 0.5°C. Two complete sets of samples were prepared, one of which was analyzed for viable CFU and injury by measuring detection time immediately following irradiation. The second set of samples, including additional samples at 0 kGy containing 2 and 6% NaCl, was temperature abused at 35°C for 20 h before analysis. The entire study was replicated twice.

Effect of adding NaCl or sucrose to fresh pork loin on the survival of s^r when irradiated

Matched pork loins were obtained 1 day post-harvest from a local abattoir. The Longissimus dorsi muscle tissue from each loin was cut from the bone, trimmed of fat, and cut into 1/2" strips. The strips were then ground through a 3/16" plate under nitrogen. Various quantities of salt or sucrose were added to the ground pork in Stomacher 400 polyethylene bags to a level of 0, 2, 4, 6, and 8% wt/wt. The additives were mixed into the samples by blending and mixing with a spatula. A mean inoculum of 8.64 $\log_{10}$ CFU/g of meat from a 16 to 18 h culture of s^r in TSB was used and mixed well by hand massaging the Stomacher bag.

TABLE 1. *Surviving CFU of S.* typhimurium *on irradiated, sterile, MDCM mixed with various amounts of NaCl.*

	Percent NaCl				
Dose kG Y	0%	2%	4%	6%	8%
	Logarithm$_{10}$ CFU/g				
0	8.65		8.61		8.70
	(9.01)	(8.91)	(8.53)	(8.24)	(8.17)
0.75		6.64		6.90	
		(8.33)		(6.14)	
1.50	5.09		6.15		6.58
	(7.88)		(6.57)		(5.97)
2.25		3.72		4.83	
		(7.31)		(3.85)	
3.00	2.08		3.18		4.14
	(2.50)		(2.33)		(3.27)

[a] Values in parentheses are those for samples that were temperature abused at 35°C for 20 h.
The reported values are the means from two independent studies.

Samples of 5.0 ± 0.05 g were placed into sterile Stomacher 400 polyethylene bags and spread over an area of approximately 10 × 10 cm. These bags were vacuum sealed. A modified response-surface central composite design, with two independent replicates, was used as illustrated in Tables 2 and 3. The samples were irradiated at 5.0 ± 0.5°C after which the CFU were determined as described above. Each experiment was replicated twice.

Pork was obtained and trimmed as above, and the fat-free meat was cut into 1/2" cubes. The cubes were frozen in dry ice and then freeze dried to a mean water content of 4.47% wt/wt. The freeze-dried pork cubes were pulverized in a bowl cutter (Model HCM 450 Hobart Food Cutter, Hobart Corporation, Troy, OH) following the procedures of Pettinati et al. *(19)* and passed through a 40-mesh stainless steel sieve under nitrogen. The powder was packaged under nitrogen and stored at -90°C until rehydrated.

In some studies the freeze-dried powdered pork was partially or totally rehydrated. The weight of the water, including inoculum, to be added to achieve the desired water fractions in the meat was calculated from the following formula:

$$W = f_d*P*x/(1-x) - f_d*P*w_d$$

W is weight of water to be added to yield weight of sample P; x is the desired fraction of water in the rehydrated sample; and f_d and w_d are the fraction of dry solids in the original meat and the fraction of water in the freeze-dried meat, respectively. The target rehydration values were 10, 25, 40, 55, and 70%; the actual values are presented in Table 4.

Freeze-dried pork was prepared as above and rehydrated with salt solutions of 0, 25, 50, 75, and 100% saturation. The percent total solids of the rehydrated meats were 38.0, 40.8, 41.3, 46.9, and 55.0, respectively. The actual ash contents presented in Table 5 indicate that salt levels of 0, 2.36, 5.23, 6.43, and 11.97% were obtained in the rehydrated pork.

Comparison of S. typhimurium *survival in NaCl or sucrose solutions of equal a_w-value*

The lack of a significant response to the presence of sucrose on meat by *S. typhimurium* during irradiation caused us to per-

TABLE 2. *Surviving CFU of s^r on irradiated, ground pork L. dorsi muscle containing various amounts of NaCl.*

	Target concentrations of NaCl				
Dose kGy	0%	2%	4%	6%	8%
	Logarithm$_{10}$ CFU/g				
0	8.71		8.47		8.46
0.72		7.32		7.60	
1.45	5.53		5.81		6.72
2.17		4.38		5.10	
2.90	1.64		3.08		3.72
4.34		0.51		0.76	
5.79	0		0		0.30

The reported values are the means from two independent studies.

TABLE 3. *Surviving CFU of s^r on irradiated, ground pork L. dorsi muscle containing various amounts of sucrose.*

	Target concentrations of sucrose				
Dose kGy	0%	2%	4%	6%	8%
	Logarithm$_{10}$ CFU/g				
0	8.81		8.74		8.60
0.72		6.97		7.46	
1.45	5.87		5.73		5.98
2.17		3.58		3.88	
2.90	2.03		2.04		2.64
4.34		0		0	
5.79	0		0		0

The reported values are the means from two independent studies.

TABLE 4. *Surviving CFU of s^r on irradiated, freeze-dried, ground pork L. dorsi muscle rehydrated to various water contents.*

	Water content and a_w values (measured)				
%	10.98	25.30	39.14	54.22	67.66
a_w	55.6	85.9	91.4	92.4	94.6
Dose (kGy)	Logarithm$_{10}$ CFU/g				
0	9.12	9.51	9.15	9.30	9.39
0.75		7.05		8.33	
1.50	7.74		7.25		7.09
2.25		4.38		mis[a]	
3.00	6.00		3.75		2.59
3.75		6.96		1.98	
4.50	3.76		0.06		0

[a] Mis: missing sample.
The reported values are the means from two independent studies.

form a direct comparison of the effects of the two humectants. Sucrose and NaCl solutions of known a_w-values were prepared in pH 7.00 0.05 M potassium phosphate buffers according to Scott *(21)*. The molal concentrations of NaCl providing a_w-values of 0.995, 0.980, 0.960, 0.940, 0.920, 0.900, and 0.850 were 0.150, 0.607, 1.20, 1.77, 2.31, 2.83, and 4.03, respectively. The molal concentrations of sucrose providing a_w-values of 0.995, 0.980,

TABLE 5. *Surviving CFU of s[r] on irradiated, freeze-dried, ground pork L. dorsi muscle rehydrated with various concentrations of NaCl.*

	Rehydration solutions percent of NaCl saturation				
% Satd.[a]	0%	25%	50%	75%	100%
% Ash	0.952	3.31	6.18	7.38	12.92
a_w	95.0	92.6	88.6	82.8	74.7
kGy	Logarithm$_{10}$ CFU/g				
0	9.22	9.02	9.18	9.01	8.77
0.75	8.16	8.32	8.37	8.31	8.13
1.50	7.00	7.68	7.65	7.63	7.09
2.25	5.00	6.18	6.44	6.56	5.97
3.00	2.95	4.96	5.36	5.57	4.61

[a] % Satd.: Percent of saturation (35.71 g/100 ml H_2O at 5°C).
The reported values are the means from two independent studies.

0.960, 0.940, 0.920, 0.900, and 0.850 were 0.272, 1.03, 1.92, 2.72, 3.48, 4.11, and 5.98, respectively. These solutions were filter sterilized. In order to minimize the effects of the inocula on the a_w of these solutions prior to irradiation, *S. typhimurium* was harvested after 16 h as above and suspended in an equal volume of Butterfield's phosphate. Aliquots of 1.5 ml were placed in Eppendorf micro centrifuge tubes and centrifuged to compact pellets from which the suspending buffer was decanted. The *S. typhimurium* cells were resuspended in aliquots of 1.5 ml of the appropriate solution of NaCl or sucrose. Preliminary experiments established that these solutions were not in themselves lethal to *S. typhimurium*. These cell suspensions in either NaCl or sucrose were irradiated to an absorbed dose of 0.8 kGy at 5°C. In order to minimize the effects of osmotic shock, serial dilutions were initiated with a 1/10 dilution in the same solution to determine the surviving CFU/ml. Measurements of detection time were also made on these cell suspensions following irradiation to allow injury to be estimated.

Statistical analysis

Responses were expressed as the logarithm of the number of CFU per g. These responses were converted into survival values, that is the logarithm$_{10}$ of the number of CFU (N) divided by the initial number of CFU (N_o) Using this format, where the logarithm of N/N_o is plotted against radiation dose, the destruction of one log of CFU (1 D_{10}) has the value of -1.0; and D_{10}-values are the negative reciprocal of the slope of the individual regression of the logarithm N/N_o plotted against radiation dose.

Radiation D_{10}-values were determined by least-squares analysis of the survival data, excluding the 0 kGy data to avoid possible shoulder effects, using the Linear Regression (REG) procedure of the Statistical Analysis System (SAS) statistical package (7, 20). Regression techniques were used to fit second-order response-surface models (4), and calculations were performed using the general linear models procedure of the SAS statistical package (7). The regressions were tested for differences by analysis of covariance.

RESULTS

Effect of NaCl content in MDCM

Adding NaCl to sterile MDCM significantly increased (p = 0.0093) the survival of *S. typhimurium* in irradiated samples (Table 1). The appropriate response surface is described by equation I (Table 7).

Control (0 kGy) samples were included at 2 and 6% NaCl to establish a baseline for the abuse study. With an inoculum of 8 log$_{10}$ CFU/g it would not be logical to expect significant multiplication to occur in the unirradiated samples during temperature abuse. It was considered essential, however, to establish that the presence of the NaCl did not significantly affect the population of *S. typhimurium* during the 20-h period of incubation. The results indicated that there was no effect on the population of *S. typhimurium* on the unirradiated MDCM and that any changes in population that might be observed would be due to the temperature abuse. When such samples were temperature abused after irradiation, the presence of salt concentrations of 4, 6, or 8% inhibited the multiplication of the surviving bacteria and may have been toxic to bacteria surviving radiation doses of 2.25 kGy or greater (Table 1).

Effects of NaCl and sucrose content in irradiated, ground pork Longissimus dorsi muscle tissue on survival of s[r]

Adding NaCl to ground, non-sterile pork *L. dorsi* muscle tissue significantly increased (p = 0.0024) the survival of *S. typhimurium* s[r] in irradiated samples (Table 2). The appropriate response surface is described by equation II (Table 7). (The statistical analysis considered only values for 3 kGy and below, as there was little survival at radiation doses greater than 3.0 kGy.)

In contrast to the results obtained by adding NaCl to ground pork *L. dorsi* muscle, adding up to 8% wt/wt of sucrose to ground pork loin did not significantly alter (p > 0.05) the survival of s[r] when the meat was irradiated (Table 3).

Effect of water content of irradiated, rehydrated, freeze-dried pork L. dorsi muscle tissue on survival of s[r]

Statistical analysis of the survival of s[r] on irradiated, rehydrated, freeze-dried pork *L. dorsi* muscle tissue over the dose range of 0 to 3.0 kGy revealed its inverse dependence (p < 0.0001) on the extent of rehydration (percent of water) and resulting a_w (Table 4, Fig. 1). The appropriate response surface is described by equation III (Table 7).

The appropriate response surface for the logarithm$_{10}$ of the number of surviving s[r] as affected by the a_w of the rehydrated pork muscle is described by equation IV (Table 7).

Effect of salt added to irradiated, rehydrated, freeze-dried pork L. dorsi muscle tissue on survival of s[r]

The survival of s[r] on irradiated, freeze-dried pork *L. dorsi* muscle tissue rehydrated with saturated and partially saturated NaCl solutions increased in a curvilinear relationship to the final salt concentration of the meat (Table 5, Fig. 2). There is some evidence from Table 5 and Fig. 2 that the highest concentration of NaCl may have been slightly toxic for the *S. typhimurium*. Analysis of variance indicated highly significant effects for the radiation dose × NaCl interaction and for the radiation dose × a_w interaction of p < 0.0001 (Equation V, Table 7) and p < 0.0025 (Equation VI, Table 7), respectively. The appropriate response surfaces for the effect of NaCl is described by equation V and VI (Table 7).

seemed unlikely that the a_w of the suspending medium was the explanation. A clue was provided when a slightly better fit for the regression was obtained by plotting survival against the molality of the suspending solution rather than its a_w. The observation of Anbar and Thomas (1) that OH radicals react with chloride ions to form Cl_2^- provides a possible explanation. In effect, the chloride ion is serving as an OH• radical scavenger and thereby provides protection to *S. typhimurium* during irradiation. Exactly the same effect was observed in our companion study of the retention of thiamin in irradiated pork. This conclusion was supported by the observed decrease in injury when NaCl was added to pork.

When we examined the effect of water content of meat on the survival of *S. typhimurium* during irradiation we found that there was a very strong correlation between reduced water content and increased survival. Because the bacterial cells were not freeze dried themselves, but rather were added to the rehydrated meat, we must conclude that the increased survival is related to the decreased availability of extracellular water to react with ionizing radiation, which would result in the death of the organism through secondary reactions. This same phenomenon occurs at very low temperatures where the mobility of the free radical is severely limited, as was discussed previously.

In a practical sense these observations provide a better understanding of the factors influencing the effectiveness of ionizing radiation for the control of foodborne pathogens. They also lead to the conclusion that ionizing irradiation of cured meat products for the control of foodborne pathogens may be less effective than might be presumed from other data.

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